DNA QUANTITATION SETUP USING THE EPMOTION

A. SCOPE

A.1 The Plexor HY System is a real-time PCR assay to determine the concentration of total human DNA and male human DNA simultaneously in one reaction. The kit contains an internal PCR control (IPC) to test for false-negative or inaccurate results that may occur in the presence of PCR inhibitors and a melt curve function to confirm that the correct product was amplified.

A.1.1 The Plexor HY System works by measuring a reduction in fluorescent signal during amplification. Amplification of each target uses only two primers, one of which contains both a fluorescent tag and a modified base. As amplification proceeds, fluorescence is reduced by site-specific incorporation of a fluorescent quencher opposite the complementary modified base. The quencher is in close proximity to a fluorescent dye located on the end of the primer, resulting in a reduction of fluorescent signal. After PCR, a melt analysis can be performed to provide an internal control for the final assay design or to expedite troubleshooting.

A.2 The quantitation setup process consists of multiple transfers of liquids containing either reagents or DNA from one place to another. By utilizing the epMotion 5075, a liquid handling robot, the incidence of human error and/or the introduction of contamination in this process can be minimized. Furthermore, automation of the quantitation setup process allows for analysts to complete other tasks while these steps are being performed.

B. QUALITY CONTROL

B.1 A lab coat, mask and protective gloves must be worn when performing this procedure.

B.2 Decontaminate the bench work area with a bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner, before and after quantitation setup.

B.3 Decontaminate the epMotion deck with Decon Quat cleaning solution followed by 70% ethanol, before and after quantitation setup.

B.4 Decontaminate the Eppendorf 24 TC racks with 70% ethanol after quantitation setup.

B.5 Decontaminate the waste container with a bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner after quantitation setup.

B.6 See DOC ID 1835 to determine reagent expiration dates.

B.7 Each new lot of Plexor HY must undergo quality control testing prior to being used for the quantitation of casework samples; see DOC ID 1784 for more information about this testing.

B.8 Each new lot of TE^{-4} must undergo quality control testing prior to being used to dilute casework samples; see DOC ID 1784 for more information about this testing.

B.9 At least one negative quantitation control must be quantitated on each quantitation plate.

B.10 Optical plates should be kept in the appropriate base at all times during plate setup and centrifugation. This limits the amount of debris introduced into the AB 7500 instrument and prevents damage to the plate wells that may interfere with the optical readings.

Quantitation setup must be performed in the pre-amplification room. Do not bring quantitation plate bases into the post amplification room.

C. SAFETY

C.1 Protective gloves, a lab coat and a mask must be worn during plate setup. Additionally, eye protection (e.g. safety glasses or a face shield) must be worn when working with any reagents or DNA extracts outside of the epMotion.

C.2 All appropriate SDS sheets must be read prior to performing this procedure.

C.3 Treat all biological specimens as potentially infectious.

C.4 Distinguish all waste as general, biohazard, or sharps and discard appropriately. The heat block of the AB 7500 can become very hot. Be careful not to touch the heating surfaces during plate loading and unloading.

D. REAGENTS, STANDARDS AND CONTROLS

D.1 Plexor HY Quantitation Kit (Promega)

D.2 Clorox Bleach Germicidal Cleaner (Decontamination)

D.3 Decon Quat (Decontamination)

D.4 70% Ethanol (Decontamination)

D.5 TE^{-4} (10 mM Tris-HCl, 0.1 mM EDTA, 1L)

Add 10 mL 1 M Tris-HCl, pH 8 and 150 µL 0.5 M EDTA to 990 mL deionized water. Store at room temperature.

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 epMotion 5075 (instrument, computer, and appropriate software)

E.1.2 epMotion dispensing tools

E.1.3 epMotion labware (thermoblock and 24TC racks)

E.1.4 AB 7500 Real-Time PCR instrument and software

E.1.5 Microcentrifuge

E.1.6 Pipettes

E.1.7 Vortexer

E.1.8 96 well-plate centrifuge

E.2 Supplies

E.2.1 epMotion supplies (epT.I.P.S. Motion 1-50 µL tips, epT.I.P.S. Motion 20-300 µL tips, and epT.I.P.S. Motion 40-1000 µL tips)

E.2.2 Sterile aerosol-resistant tips

E.2.3 Microcentrifuge tubes racks

E.2.4 AB optical 96-well plates

E.2.5 AB optical adhesive covers

E.2.6 Adhesive seal applicator

E.2.7 96-well plate base

E.2.8 Disposable gloves

E.2.9 Mask

E.2.10 Lab coat

E.2.11 Eye protection (e.g. safety glasses, face shield)

E.2.12 DNA Analysis Workbook (optional)

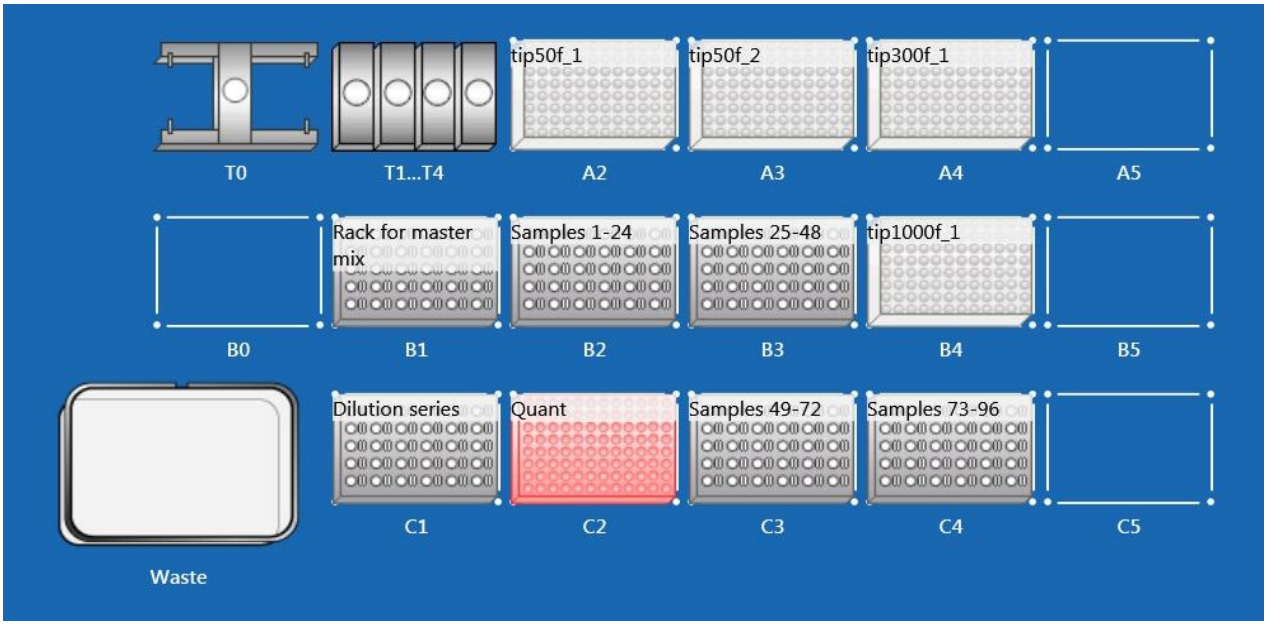
F. PROCEDURES

F.1 Thaw the Plexor HY Male Genomic DNA Standard (50 ng/µL) overnight at approximately 4°C. After initial thawing, store at approximately 4°C.

F.2 Open the Eppendorf eBlue software.

F.3 Select **Application Editor** from the main menu, from the **DNA** user tab choose the **Quantitation** folder and then select the **Quantitation_Setup** method.

F.4 Prepare the epMotion worktable using the following diagram and instructions for guidance:



F.4.1 Place epT.I.P.S. Motion 1-50 µL tips in positions A2 and A3 (A2 and A3 are denoted as "tips50f_1" and "tips50f_2", respectively), epT.I.P.S. Motion 20-300 µL tips in position A4 (A4 is denoted as "tips300f_1"), and epT.I.P.S. Motion 40-1000 µL tips in position B4 (B4 is denoted as "tips1000f_1") as shown above. All tip boxes should be placed on the worktable so that the manufacturer's label is on the left side. The robot can use two types of tip versions. If using the older version the operator **MUST VERIFY <u>that the box has sufficient tips for pipetting and has no tips out of place from the first tip position</u>**.

F.4.2 Ensure that the epMotion dispensing tools needed for this protocol, i.e. the TS 50 single channel, TS 300 single channel, and TS 1000 single channel tools, are in place on the worktable; these tools can be placed in any sequence on the tool holders in position T1…T4.

F.4.3 Place Eppendorf 24 TC racks in positions B1, B2, B3, C1, C3, and C4 on the worktable; these are denoted as "Rack for master", "Samples 1-24", "Samples 25-48", "Dilution series", "Samples 49-72" and "Samples 73-96", respectively.

F.4.4 Place a thermoblock in position C2 (denoted as "Quant").

F.4.5 Place an empty, closed dolphin tube in 24 of the rack in position B1 ("Rack for master") on the worktable.

F.4.6 Place empty, closed QIAcube elution tubes in 13, 14, 15, 16, 17, and 18 of the rack in position C1 ("Dilution series") on the worktable.

F.5 Add the necessary reagents to the epMotion worktable by following the below instructions:

F.5.1 Vortex the Plexor HY Male Genomic DNA Standard for at least 5 seconds and place this closed tube in 6 of the rack in position C1 on the worktable; see pg 6 for photo. This tube must contain at least 15 µL of the Standard. The epMotion quantitation protocol

creates a serial dilution from this 50 ng/µL standard, creating the following standard concentrations: 10 ng/µL, 2 ng/µL, 0.4 ng/µL, 0.08 ng/µL, 0.016 ng/µL, and 0.0032 ng/µL.

F.5.2 Thaw the Plexor HY 2X Master Mix, Plexor HY 20X Primer/IPC Mix, and amplification grade water at room temperature; the amplification grade water may be stored in the refrigerator.

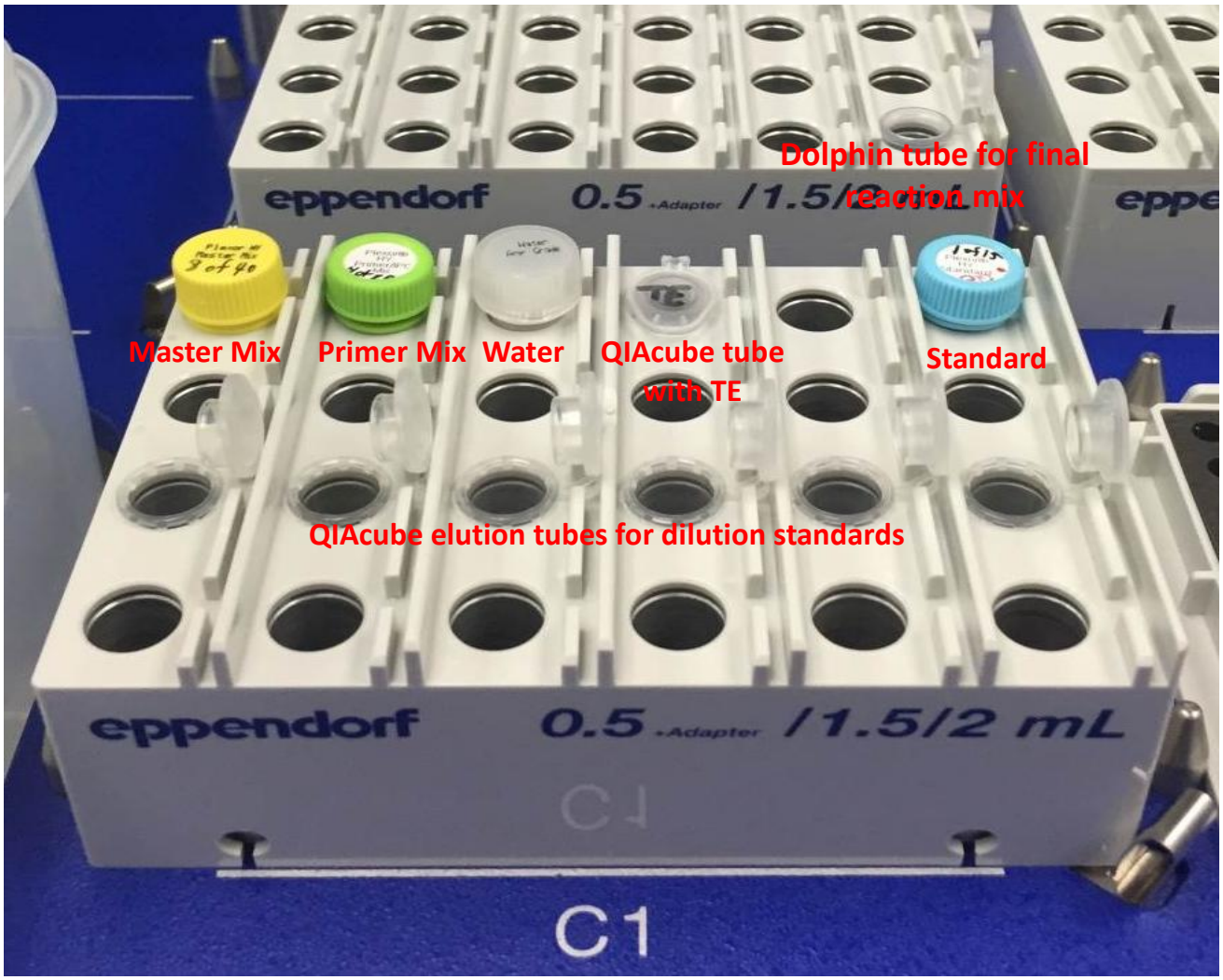
F.5.2.1 Briefly vortex the Plexor HY 2X Master Mix and Plexor HY 20X Primer/IPC Mix for 3–5 seconds to mix. **Do not** centrifuge after vortexing, as this may cause the primers to be concentrated at the bottom of the tube.

F.5.2.2 Determine the number of reactions to be setup including two no-template controls (NTCs). Add additional reactions to this number to compensate for loss during pipetting (6 extra samples are needed for a full epMotion quantitation plate, i.e. 80 sample extracts, 14 Quant Standards, and 2 NTC's). Ensure that the volume in each of the reagent tubes is sufficient; see below chart for the volume of each component needed per reaction.

Component	Volume (Per Reaction)
Plexor HY 2X Master Mix	10 µL
Water, Amplification Grade	7 µL
Plexor HY 20X Primer / IPC Mix	1 µL
Final volume	18 µL

Note: If a full plate is utilized with 6 extra samples worth of reagents, 1020 µL of Plexor HY 2X Master Mix is required. The instrument is only able to pipette up to 1000 µL. Therefore 20 µL of Plexor HY 2X Master Mix must be added to the final Quant Master Mix reagent tube off-deck, in addition to programming the robot to add the initial 1000 µL.

F.5.2.3 Place the closed Plexor HY 2X Master Mix tube in 1, closed Plexor HY 20X Primer/IPC Mix tube in 2, closed amplification grade water tube in 3, and closed 150ul of TE^{-4} in 4 of the rack in position C1 ("Dilution Series" rack); see photo below.

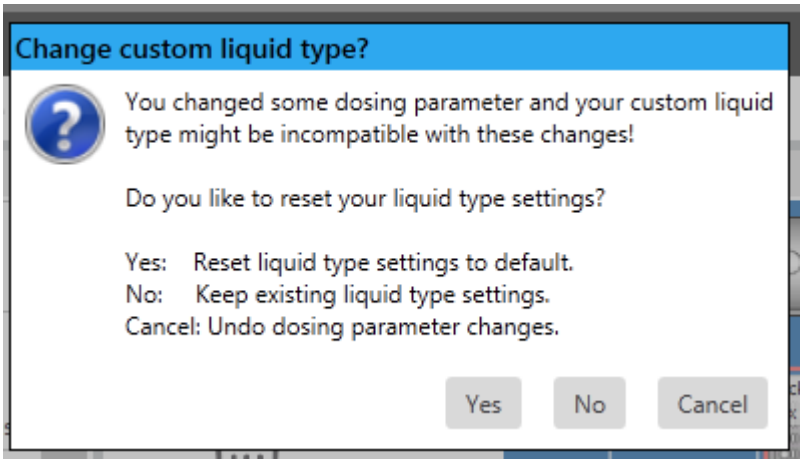


F.6 The **Quantitation_Setup.dws** method must be modified with every quantitation plate performed; this modification is necessary to account for the variable nature of the number of sample extracts.

F.6.1 The **Quantitation-Setup.dws** method is "read only"; therefore, click the **Save As Icon** and name the application with your quantitation run name so that the following method modifications can be made.

F.6.2 Click on the **switch to procedure button** and then click on **Step 10 - Reagent Transfer**. Using your quantitation setup plate map and associated reagent calculations, enter the volume of Plexor HY Master Mix to pipette in the **Volume** location. If this number

is ≥ 300 µL change the **Pipet Tool** in this reagent transfer step from the TS_300 to the TS_1000. The following message will appear when the volume and the tool are changed; select **No**. Click the **Save Icon**.



F.6.3 Click on **Step 12 - Reagent Transfer**. Enter the volume of Plexor HY Primer to pipette in the **Volume** location. If this number is ≥ 50 µL change the **Pipet Tool** in this reagent transfer step from the TS_50 to the TS_300. Click the **Save Icon**.

F.6.4 Click on **Step 14 - Reagent Transfer**. Enter the volume of water to pipette in the **Volume** location. If this number is ≥ 300 µL change the **Pipet Tool** in this reagent transfer step from the TS_300 to the TS_1000. Click the **Save Icon**.

F.7 Place open sample extract tubes in racks B2 (Samples 1-24), B3 (Samples 25-48), C3 (Samples 49-72), and C4 (Samples 73-80), as necessary. Sample extract evaporation can be limited by storing these racks in the refrigerator prior to use.

F.7.1 The racks are numbered for sample order; sample extracts 1-6 should be placed in the first row, sample extracts 7-12 in the second row, and so on.

Note 1: *Sample extracts must be in QIAcube elution tubes or regular manual extraction tubes (these manual tubes have holes in their snap caps). Dolphin tubes fit very tightly in the Eppendorf 24 TC rack and therefore, often get stuck; if a sample has been concentrated into a dolphin tube prior to quantitation, transfer this sample extract into one of the aforementioned suitable tubes prior to using the epMotion.*

Note 2: *The closed sample extract tubes can be placed on the epMotion deck at any point prior to step F.8; however, the tubes should not be opened until this step (F.7) to avoid evaporation.*

F.8 Open all tubes containing reagents, water, and TE^{-4} on the epMotion worktable and open the empty dilution series and Plexor HY final reaction mix tubes; alternatively, the caps can be removed from the reagent tubes.

F.9 Place an empty 96-well plate on the thermoblock in position C2 ("Quant").

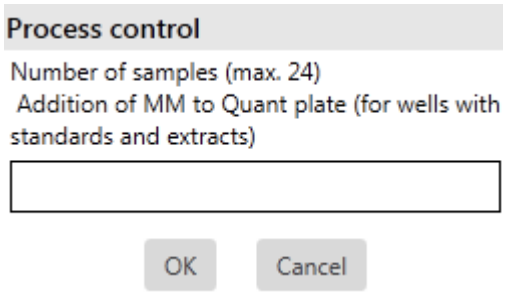
F.10 Close the front hood of the epMotion.

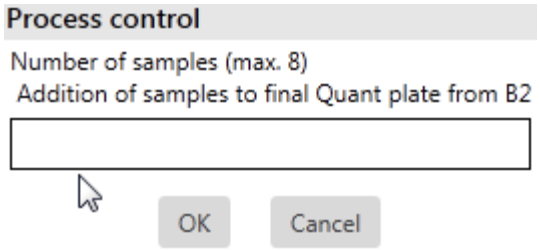
F.11 Select the **Run Icon**

F.12 Ensure that compatible devices are selected and that device 5075ZN301615 is highlighted, click **Next**.

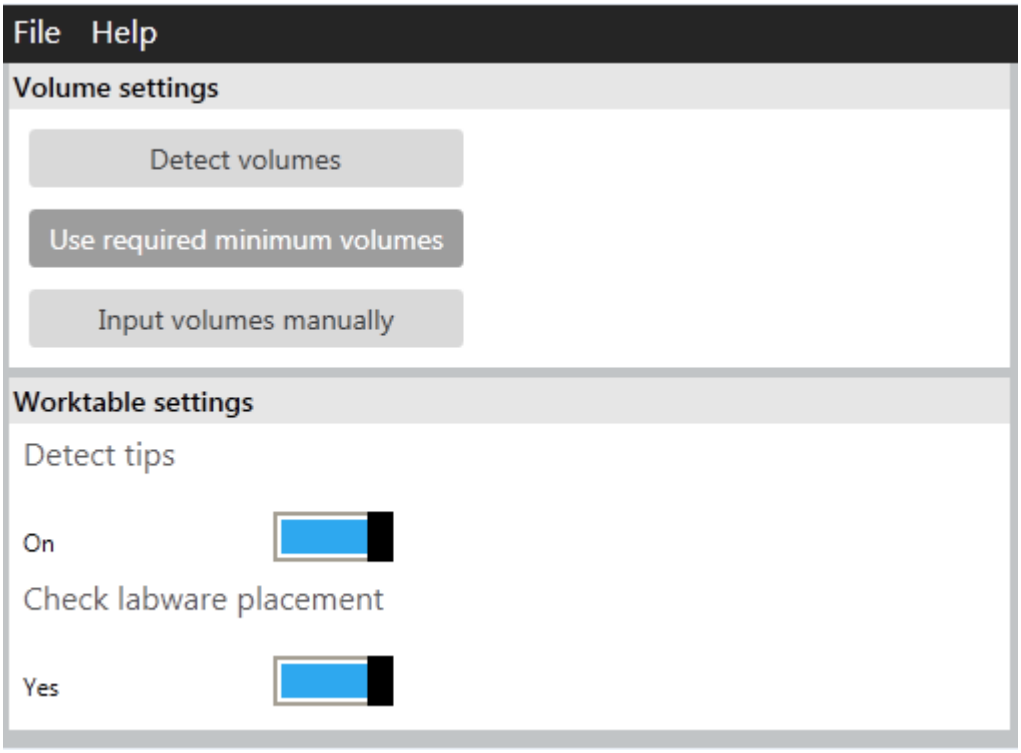
F.13 You will be prompted to input the number of wells the master mix will be added to the quant plate enter the information and click **OK.** Then you will be prompted to input the number of samples from each sample rack, enter this information and click **OK** each time.

Example: If 40 sample extracts were to be quantitated, "56" would be entered in the first prompt for total wells to add the master mix to, and "24" would be entered in the next prompt, "16" would be entered in the next prompt, and "0" would be entered into the next two prompts.





F.14 On the following screen ensure that **Use required minimum volumes**, **Detect tips** and **Check labware placement** are selected click **Run**.



F.15 The method will start and the display will switch to the Control tab. The progress and current status of the method will be displayed. A message will appear when the method has been completed.

***Note**: Quantitation setup of approximately 70 wells using the robot takes approximately 45 minutes.*

F.15.1 To stop the method before it is complete, click the **Pause** icon in the Control tab or lift the front hood. Then click the **Cancel** icon to abort the method; the front hood must be down to abort the method. Alternatively, after stopping the method, click the **Run** icon to continue the method.

F.15.2 During the initial labware check if you do not have samples in racks B3, C3, and C4 the software will prompt that those areas are not detected. Choose **Ignore** for each prompt.

F.16 After completion of the method, select **OK** and then **exit.** Select file **Exit to Start Screen** and select **Log Viewer** select your log file by finding the file with the appropriate date and time.

Select the **Print icon** and then choose the **PDF** button save the file as a PDF document. This file should be saved in the appropriate analyst's casework folder on the I drive. A run completed without errors will have "Program ended successfully" on the last line of the log file.

F.17 Open the front hood and remove the 96-well plate and place it into a plate base. Seal the plate with an optical adhesive cover using the plate cover applicator.

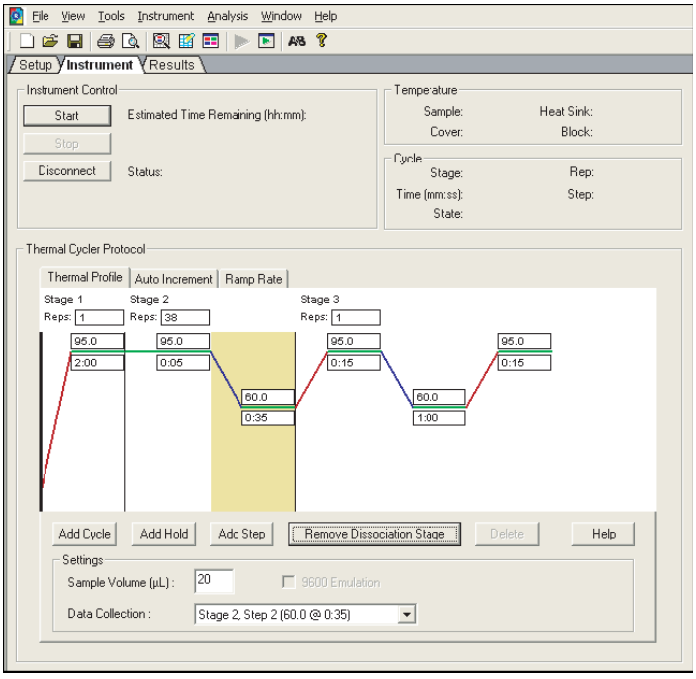
F.18 Cap the Plexor HY 2X Master Mix, Plexor HY 20X Primer/IPC Mix, amplification grade water, and Plexor HY Male Genomic DNA Standard on the epMotion deck.

F.19 Centrifuge the plate briefly to collect the contents of the wells at the bottom. The plate is ready for thermal cycling. Protect the plate from extended light exposure or elevated temperatures before cycling. Handle the plate by the edges, and avoid touching the bottom of the plate.

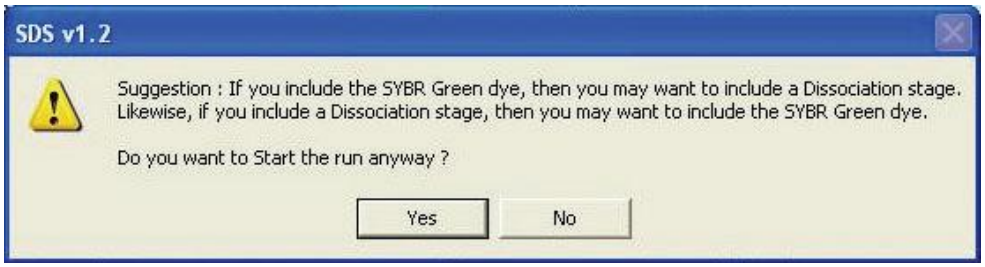
F.20 Position the plate in the instrument thermal block so the Well A1 is in the upper-left corner. The notched corner of the plate is in the upper-right corner.

F.21 In the SDS software open the plate document that was set up for a Plexor HY run. Uncheck wells that are not being used. Save the file as an .sds document. The following message will appear when saving the document. Click "Yes".

Select the **Instrument** tab. The following cycling parameters are displayed:



F.22 Click **Start.** The following message will appear when starting the instrument. Click "Yes".



F.23 Prepare the epMotion worktable for the next user:

F.23.1 Remove the Plexor HY 2X Master Mix, Plexor HY 20X Primer/IPC Mix, amplification grade water, and Plexor HY Male Genomic DNA Standard tubes from the epMotion deck.

F.23.2 Discard any unused Plexor HY reaction mix and TE^{-4}.

F.23.3 Decontaminate any used epMotion labware (i.e. Eppendorf 24 TC racks and 96-well thermoblock) with 70% ethanol; store this labware in the refrigerator.

F.23.4 Empty the waste container and decontaminate it with a bleach-based cleaner, e.g. Clorox Bleach Germicidal Cleaner.

F.23.5 Decontaminate the epMotion deck with Decon Quat cleaning solution followed by 70% ethanol.

G. INTERPRETATION GUIDELINES

G.1 See Analysis of Plexor HY Data procedure.

H. REFERENCES

H.1 Eppendorf epMotion 5075 with Integrated PC and epBlue Operating Manual, 2008.

H.2 EpMotion 5075 with Integrated PC and epBlue Operating Manual, Eppendorf, Hamburg, Germany 2008.

H.3 Pre-Amp epMotion Validation Binder 1, Quantitation_Setup.dws method.